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Resource Allocation and Cultivar Stability in Breeding for Fusarium Head Blight Resistance in Spring Wheat

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ABSTRACT

The development of wheat (*Triticum aestivum* L.) cultivars resistant to Fusarium head blight (FHB) (caused by *Fusarium graminearum* Schwabe) requires screening methodologies that accurately characterize reaction to this disease. The objectives of this study were (i) to characterize the stability of cultivars for their FHB reaction and (ii) to define an optimum resource allocation for FHB evaluation. Fourteen cultivars were evaluated in FHB-screening nurseries at two locations across a 4-yr period. Field data were used to calculate disease incidence (INC) as the frequency of symptomatic spikes and disease index (DIS) as the mean disease score of all spikes. FHB reaction also was evaluated on harvested grain as percent visually scabby kernels (VSK) and deoxynivalenol (DON) concentration. Significant differences among cultivars for all FHB parameters were found in each environment. Pearson correlation coefficients among FHB parameters were positive and highly significant, ranging from 0.32 between INC and DON to 0.72 between INC and DIS. Spearman rank correlation coefficients for yearly cultivar rankings and Kendall's coefficient of concordance were high, indicating similarity of the rankings of the tested cultivars in different environments. Visually scabby kernels was the FHB parameter with highest similarity for cultivar ranking across environments. Most of the cultivars, including susceptible ones, expressed stability for FHB reaction. Optimum resource allocation for DIS was most affected by the number of environments with three being the minimum to accurately characterize a genotype's resistance level. Using more than three replications or scoring more than 10 spikes per plot had little practical value in characterizing FHB reaction.

Fusarium head blight, caused primarily by *Fusarium graminearum*, is an important disease throughout major spring wheat-production regions of the USA, Canada, and other parts of the world. Even low disease levels can result in loss of grain yield and quality, accumulation of mycotoxins (primarily deoxynivalenol), and reduction in seed quality (Snijders, 1990; Parry et al., 1995; McMullen et al., 1997). Researchers often suggest that disease-resistant cultivars represent the best method of control (Snijders, 1990; Wiersma et al., 1996; McMullen et al., 1997; Campbell and Lipps, 1998; Yang et al., 1999). Resistance expression often differs among environments (Parry et al., 1995; Campbell and Lipps, 1998; Groth et al., 1999); consequently, developing cultivars with FHB resistance requires experimental designs and strategies that consistently discriminate among genotypes.

Disease development and evaluation of FHB is complex and is readily altered by environment (Parry et al., 1995; van Eeuwijk et al., 1995; Mesterházy, 1995, 1997; Groth et al., 1999). Using a single trait to characterize FHB resistance could be misleading because of the complexity of plant response to the disease. Many types and components of disease reaction have been described to help understand differences in disease response (Mesterházy, 1995; Parry et al., 1995; Dill-Macky, 2003). Often, the types and components relate to different aspects of FHB development. Single spikelet inoculations are used to evaluate disease spread in the spike (Bai and Shaner, 1996; Campbell and Lipps, 1998; Yang et al., 1999); however, their utilization is often limited by the labor required to inoculate and assess host reaction(s). Infection incidence and disease severity measure the frequency and degree of colonization of the spike, respectively, and are common measures of disease (Parry et al., 1995; Dill-Macky, 2003). Disease-affected grain is often quantified by percentage of visually scabby kernels (Jones and Mirocha, 1999) and deoxynivalenol (DON) content (Tacke and Casper, 1996; Mirocha et al., 1998). Yield reduction attributable to the disease is sometimes determined (Mesterházy, 1995), and ergosterol analysis has been used to quantify fungal biomass (Miller et al., 1985), but these measures of evaluation can be expensive.

Few studies have been reported that can help assess the efficient allocation of environments, replicates, and within plot subsampling for FHB evaluation. Campbell and Lipps (1998), working with winter wheat, used estimates of variance components to guide screening-nursery experimental design. They observed the largest reduction in genotype standard error through addition of screening environments. Mesterházy (1997) suggested that genotypes be evaluated for at least 2 to 3 yr (environments) before drawing conclusions regarding their relative reaction to this disease. The stability of a genotype's reaction across different FHB screening environments is also important. If susceptible genotypes are less stable in their reaction, as Mesterházy (1995) suggests, more testing will be required to characterize them properly. The objectives of this study were to characterize

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Abbreviations: DIS, disease index; DON, deoxynivalenol; FHB, Fusarium head blight; INC, incidence; VSK, visually scabby kernels.

the stability of cultivars for their FHB reaction and to define an optimal resource allocation for FHB evaluation.

MATERIALS AND METHODS

Plant Materials

Fourteen commercial wheat cultivars originating from Minnesota, North Dakota, South Dakota, and Manitoba, Canada, were used in this study. The cultivars encompass a broad range of response to FHB from resistant to susceptible, and some are commonly used as check cultivars in screening nurseries used by the Univ. of Minnesota wheat-breeding program. The cultivars were BacUp, Forge, Gunner, HJ98, Ingot, Marshall, McVey, Norm, Oxen, Roblin, Russ, Verde, Wheaton, and 2375. In Minnesota, the cultivars head on average from 50 to 58 d after planting. These cultivars were grown in FHB nurseries at St. Paul and Crookston, MN, from 1999 to 2002 in a randomized complete-block design, with three replicates per location per year. In each year, 18 to 22 other genotypes were grown in the same trial, but their results are not reported here because they were not grown in all four years. At St. Paul, plots were sown on 29 April 1999, 26 April 2000, 26 April 2001, and 30 April 2002. The insecticide disulfoton, *O,O*-dimethyl *S*-2-ethylthioethyl phosphorodithioate, (0.56 kg ha^{-1} a.i.) was applied at the 3- to 5-leaf stage in 2000 to control aphids (Aphididea). At Crookston, plots were sown on 3 May 1999, 28 April 2000, 8 May 2001, and 17 May 2002. At both locations, the row spacing was 0.3 m with a plot consisting of a single 2.4-m row and a seeding rate of 110 kg ha^{-1}. Fertilizer and herbicide were applied according to standard agronomic practices at each location. Cultural practices and disease inoculations were implemented in a manner sufficient for development of visible disease symptoms 2 to 3 wk after plant heading on all entries.

Fusarium Inoculation

St. Paul—Macroconidial Inoculum

Thirty to 36 *F. graminearum* isolates were used as inoculum at St. Paul. Isolates were obtained from symptomatic spikes collected from commercial wheat fields at various Minnesota locations. Mung-bean agar was prepared and used as a substrate to grow *F. graminearum* cultures following Dill-Macky (2003). Macroconidia were rinsed from the culture surface with water, and aliquots at 8×10^5 macroconidia mL^{-1} were prepared and stored frozen (−20°C).

Inoculum (1×10^5 macroconidia mL^{-1} and 2 mL L^{-1} polyoxyethylenesorbitan monolaurate [Tween 20; ICI, Fair Lawn, NJ]) was applied to the spikes at the onset of anthesis with a CO_2–powered backpack sprayer equipped with a single TeeJet Flat fan nozzle no. ss80015 (Teejet Agricultural Spray Products, Wheaton, IL). Pressure was adjusted to 2.8 kg cm^{-2}, and plots were sprayed evenly at a rate of 30 mL m^{-1} of row, approximately 7×10^6 macroconidia $plot^{-1}$. The sprayer allowed plots to be inoculated separately. Plants were inoculated for the first time when anthers were beginning to extrude from spikes. A second inoculation was applied to each plot three or four days after the first inoculation. Each plot was inoculated three times in 1999 and twice in 2000, 2001, and 2002. The nursery was misted eight times during a daily cycle with an automatic irrigation system. Misting was initiated with the first inoculation and continued for 26 d in 1999 and 18 d in 2000, 2001, and 2002. In 1999, plots were misted for 20 min every 3 h, except in the afternoon and evening when misting duration was increased to 30 min. This provided approximately 12.7 mm d^{-1} of water. Misting-cycle duration was reduced to 16 min in 2000, 2001, and 2002, providing approximately 7.6 mm d^{-1} of water.

Crookston—Colonized-Grain Inoculum

At Crookston, inoculum was prepared from 12 isolates obtained from infected wheat at various locations in the Red River Valley in 1994. Maize (*Zea mays* L.) kernels were autoclaved and colonized by *F. graminearum* following Dill-Macky (2003). Grain inoculum that was spread at the jointing stage was prepared up to 6 wk in advance, dried, and stored at room temperature. Freshly colonized grain (that would develop mature perithecia in approximately 14 d) was used for subsequent inoculations.

The nursery was inoculated by uniformly spreading *Fusarium*–colonized kernels on the soil surface at 100 kg ha^{-1} 27 d before mean heading date in 1999, and 24 d prior in 2000, 2001, and 2002. The interval between rainfalls was seldom more than 2 d during the 2 wk after inoculation in 1999, so initiation of mist irrigation was delayed until 15 d post-inoculation. At that time, the nursery was misted from 2100 to 0700 h for 10 min of every 80-min cycle (about 6.3 mm d^{-1} of water). Misting was stopped 27 d post-heading. In 2000, 2001, and 2002, mist irrigation was started 4 d after inoculation. The nursery was initially misted from 1700 to 1000 h for 10 min of every 90-min cycle. Misting was reduced to 2400 to 0800 h beginning 3 d post-heading, and continued for another 20 d. The two schedules provided approximately 9.4 and 4.5 mm d^{-1} of water, respectively.

Data Collection

Disease Evaluation

Heading date in all environments was recorded as the first date when one half or more of primary spikes had emerged. Visual disease scores (0–5) representative of the level of infection were assigned to dominant spikes (primary tillers) from 20 arbitrarily selected plants per plot. Spikes within 0.3 m of the row's end were not scored. In general, a spikelet was considered infected if any glumes, lemmas, and/or palea were visibly necrotic. The scores were assigned as follows: 0—no symptomatic spikelets, 1—one symptomatic spikelet, 2—two symptomatic spikelets or occasionally three if some spikelets contained florets that appeared unaffected by disease, 3—three to eight symptomatic spikelets, 4—more than 50% of the spike symptomatic and at least one unaffected spikelet, and 5—all spikelets symptomatic.

Spikes generally had 15 to 17 spikelets; accordingly, these scores represented disease symptoms in approximately 0, 6, 16, 35, 65, and 100% of the spike, respectively. The scores were used to calculate the following two variables for each plot: disease incidence (INC) recorded as percentage of the frequency of symptomatic spikes (scores 1–5) and disease index (DIS)—mean score of all spikes (scores 0–5). Spikes were assessed at late grain-filling, when healthy spikes were still green and not senescent. At St. Paul, the scoring date was adjusted relative to inoculation date; plots were scored 19 d after their respective first inoculation dates. Two scoring dates were used for 1999 at Crookston; the earlier heading plots were scored 6 d before later heading plots, averaging 28 d after heading for each group. All plots were scored 23 d after the nursery's mean heading date at Crookston in 2000, 2001, and 2002.

Visually Scabby Kernels

Post-harvest examination of grain samples following combine-harvesting (1999–2001) or hand-harvesting of a 30-spike

sample (2002) was done for each plot. Because FHB reduces kernel weight and density (Wiersma et al., 1996), during mechanical threshing the air flow over the grain sieves was reduced until essentially no FHB-affected kernels were being lost from the grain sample. The threshed samples often contained substantial nongrain material that was removed by various mechanical and manual techniques. Percentage of visually scabby kernels (VSK) was assessed following Jones and Mirocha (1999).

Deoxynivalenol Analysis

Samples from the three replications of each cultivar were bulked following VSK determination and ground for 2 min with a Stein Laboratories Mill (model M-2, Stein Laboratories Inc., Atchison, KS). Sample extraction and clean-up procedures were based on the methods of Tacke and Casper (1996) and Mirocha et al. (1998) with some modifications. A 4-g sample was extracted with 16 mL acetonitrile/water (84:16 v:v) extraction buffer. The sample was placed on a rotary shaker for 1 h; 4 mL of extract was passed through a column packed with C18 and aluminum oxide. One milliliter of filtrate was evaporated to dryness under nitrogen and derivatized by the silylating reagent (TMSI/TMCS 100:1, Pierce Chemical Co., Rockford, IL) for GC/MS analysis (Shimadzu GC–MS-QP2010, Shimadzu Corporation, Kyoto, Japan). Selected ion monitoring (SIM) was used for GC/MS analysis with fragment ion (m/z value) of 235.10 as target ion and 259.10 and 422.10 as reference ions. A set of 10 standards ranging from 25 ng g^{-1} to 16 μg g^{-1} was interspersed among the samples being analyzed. A 6-point standard curve was used to cover 25 ng g^{-1} to 1 μg g^{-1}, and the 10-point curve was used to calculate higher concentrations.

Data Analyses

Cultivars and locations were considered fixed effects. Years, replicates, and plants within plots were assumed to be random effects. Analyses of variance to compare cultivar means were conducted on INC, DIS, VSK, and DON. For DON laboratory analysis, we bulked the three replications for each environment; therefore, the analyses of variance had the sources of variation as cultivars, environments, and cultivar × environment interaction; the latter was the error term. We consider environments random effects as they arise from the combinations of locations by years, which are fixed and random, respectively. At each environment, Pearson correlation coefficients were calculated on an entry mean basis to assess the relationships among heading date, INC, DIS, VSK, and DON. Spearman rank correlation coefficients between the cultivars' yearly ranks and Kendall's coefficient of concordance (W) were estimated, according to Hühn (1996), to evaluate the similarity of cultivar rankings for each FHB parameter in different environments. Kendall's coefficient of concordance is defined as the ratio of the observed variance of the rank sums and the maximum variance of the ranks. It is equivalent to the mean of all Spearman rank correlation coefficients for all possible pair-wise comparisons of the rankings of the genotypes in different environments (Hühn, 1996). In each environment, ranking of the 14 cultivars was conducted per replication. For each parameter, Rank 1 was assigned to the cultivar with the lowest value and rank 14 assigned to the cultivar with the highest value. Estimates of pooled-error mean squares, within-plot variances, and plot-to-plot variances were obtained for DIS data following analyses of variance models described by Fehr (1987). Homogeneity of error variances was tested according to Bartlett (Steele and Torrie, 1980). Analyses of data were conducted using PROC CORR, PROC GLM, and PROC MIXED (SAS 8.1, SAS Institute Inc, 2001).

Stability Analyses

The stability of cultivars for the two FHB parameters, DIS and VSK, was assessed by considering location–year combinations as eight environments (4 yr × 2 locations). Analyses of variance were conducted to obtain the effects of cultivars, environments, and the cultivar × environment interaction (C × E). The environmental sum of squares was partitioned into linear regression and residual and the C × E interaction sum of squares was partitioned into heterogeneity of regressions and residual according to Freeman and Perkins (1971). For each cultivar, four stability parameters were estimated: regression coefficient b_i (Finlay and Wilkinson, 1963), deviation from regression parameter δ_i^2 (Eberhart and Russell, 1966), and stability variances σ_i^2 and s_i^2 (Shukla, 1972). The regression coefficient b_i and δ_i^2 were determined from the regression of each cultivar's within-environment means on an environmental index (Eberhart and Russell, 1966). These estimates are defined from the model:

$$Y_{ij} = \mu_i + b_i I_j + \delta_{ij}$$

where Y_{ij} is the mean of the ith cultivar in the jth environment, μ_i is the ith cultivar's mean, regression coefficient b_i represents the ith cultivar's response to varying environments, environmental index I_j is calculated as the overall cultivar mean within an environment minus the grand mean, and δ_{ij} is the deviation from regression of the ith cultivar in the jth environment. Estimates of Shukla (1972) stability variances $\hat{\sigma}_i^2$ for σ_i^2 and $\hat{s}_i^2$ for s_i^2 were obtained as from the models described below.

$$\hat{\sigma}_i^2 = \frac{1}{(s-1)(t-1)(t-2)}\left[t(t-1)\sum_j (u_{ij} - \overline{u_{i.}})^2 - \sum_i\sum_j (u_{ij} - \overline{u_{i.}})^2\right]$$

where $u_{ij} = \overline{y_{ij}} - \overline{y_{.j}}$ and $\overline{u_{i.}} = \sum_j u_{ij} \Big/ s$

$$\hat{s}_i^2 = \frac{t}{(s-2)(t-2)}\left[S_i - \sum_i \frac{S_i}{t(t-1)}\right]$$

where $S_i = \sum_{j=1}^{s} (u_{ij} - \overline{u_{i.}} - \hat{b}_i z_i)^2$, $\hat{b}_i$ is the estimated regression coefficient for the ith genotype, the covariate z_j (the environmental index) is the deviation of the jth environment from the overall mean, t is the number of genotypes, and s is the number of environments. Estimates of stability variances were obtained by a computer program provided at no charge by Kang (1989).

Predicted Genotype Standard Error and Least Significant Difference (LSD)

Genotype standard errors and least significant difference calculations for various combinations of the number of spikes $plot^{-1}$ evaluated, replications, and environments were calculated for the DIS parameter because this is the mostly widely used FHB parameter assessed among wheat breeders. Standard errors were calculated as $[(s_\varepsilon^2 + rs_{CE}^2)/re]^{1/2}$, where r = number of replications, e = number of environments, s_ε^2, the plot error variance = $s_w^2/n + s_b^2$, where s_w^2 is the within-plot variance with n spikes per plot evaluated, and s_b^2 is between plot variance, and s_{CE}^2 is the estimated cultivar × environment variance obtained by the model described by Freeman and Perkins (1971). Using this equation, s_ε^2 is expressed relative

to plot mean. Predicted $LSD_{0.05}$ for various levels of subsampling and replication, and various numbers of environments were calculated as $LSD = t_{(0.05,df)} \times [2 \times (s_\varepsilon^2 + rs_{CE}^2)/re]^{1/2}$ where df = degrees of freedom for pooled error, and other terms as described above (Fehr, 1987).

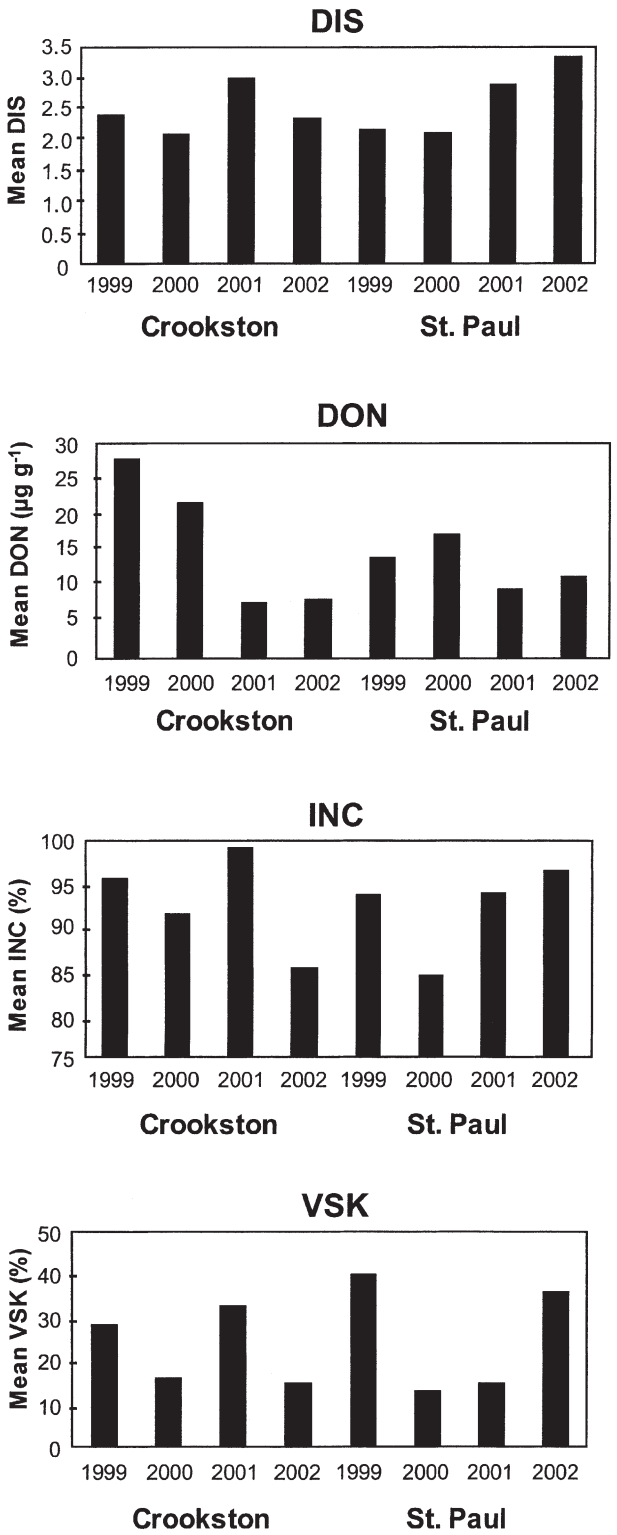

Fig. 1. Environment mean values over replications and cultivars for Fusarium head blight incidence (INC), disease index (DIS), visually scabby kernels (VSK) and deoxynivalenol (DON) at two locations, Crookston and St. Paul, MN, from 1999–2002.

RESULTS AND DISCUSSION

Disease levels varied across years and locations (Fig. 1), but disease pressure was sufficient to evaluate cultivars for FHB resistance in all environments. Table 1 contains a summary of the mean cultivar values across eight environments for INC, DIS, VSK, and DON. In each environment, significant differences ($P \leq 0.05$) among cultivars were found for all FHB parameters measured, indicating that the cultivars represented a wide range of reaction to FHB.

Pearson's correlation coefficients among five FHB parameters, including heading dates, are summarized in Table 2. Heading date had low correlation coefficients with DIS and VSK. The correlation coefficient between heading date and INC was low but significant at the 0.01 probability level ($r = 0.18$). In spite of the significance of the correlation between heading date and INC, this explained only 3.2% of the variation between the two traits, which is not very useful to plant breeders from a practical standpoint. We expected low correlation coefficients between heading dates and FHB parameters because inoculation procedures were developed to minimize the influence of heading date on the cultivars' FHB reaction. At St. Paul, we inoculated each plot at anthesis (approximately 3 d after heading), and at Crookston, the nursery was managed to produce ascospores during the range of heading dates. Correlations among the four FHB parameters were generally high and significant. Significant correlations among these traits were reported in other studies (Groth et al., 1999; Mesterházy, 1995).

Yearly rankings of cultivars for their FHB response were similar at both locations, Crooskton and St. Paul (Table 3). VSK was the parameter for which cultivar rankings were the most similar among environments. The mean values for Spearman rank correlation coefficients for VSK were 0.85 and 0.64 for Crookston and St. Paul, respectively (Table 3). Kendall's coefficient of concordance for VSK at Crookston and St. Paul were 0.85 and 0.70, respectively. From a breeder's point of view, it is desirable to have a maximum association ($W = 1$) between the rankings at different environments, which

Table 1. Cultivar means for FHB incidence (INC), disease index (DIS), visually scabby kernels (VSK), and deoxynivalenol (DON) across eight environments.

Cultivar†	INC	DIS	VSK	DON
	%	0–5	%	µg g^{-1}
BacUp	76	1.4	9.6	5.3
Ingot	84	1.9	11.7	6.6
Forge	81	1.9	18.9	7.2
2375	89	2.0	16.5	8.6
Gunner	92	2.1	13.3	8.1
McVey	94	2.1	19.8	12.6
Russ	90	2.2	24.5	10.6
Verde	98	2.5	23.5	15.0
HJ98	96	2.7	25.4	9.8
Marshall	99	2.7	27.6	18.9
Oxen	98	2.9	28.8	10.9
Norm	99	3.1	44.1	36.5
Roblin	99	3.4	30.4	12.7
Wheaton	99	3.8	49.8	35.9
Mean	92.0	2.5	24.6	14.2
LSD(0.05)	5.2	0.3	4.2	8.0

† Cultivars are ordered from lowest to highest mean DIS.

Table 2. Pearson correlation coefficients† among heading date (HD) and four parameters of FHB resistance, incidence (INC), disease index (DIS), visually scabby kernels (VSK), and deoxynivalenol (DON) using 14 cultivars in eight environments (n = 112).

	INC	DIS	VSK	DON
HD	0.18**	0.10	0.04	0.07
INC		0.72***	0.50***	0.32***
DIS			0.62***	0.37***
VSK				0.45***

** Significant at 0.01 probability level.
*** Significant at 0.001 probability level.
† Correlations among DON and other parameters were obtained on environment means (n = 112).

implies identical rank orders of genotypes in each environment tested. For a large W, it would be expected with a high chance that if this set of genotypes were evaluated in another environment, the rankings would be similar or even the same as in other environments (Hühn, 1996). Wheat breeders should take advantage of the similarity of cultivar rankings in different environments using VSK (and the relatively low cost of it compared with DON) and incorporate VSK in their selection procedures. Similarity of yearly cultivar rankings on the basis of DIS and DON were also high, with mean Spearman rank correlation values of 0.69 for both parameters for Crookston and 0.54 and 0.66, respectively, for St. Paul (Table 3).

Yearly cultivar rankings for their FHB reactions on the basis of INC were less similar among environments (r_s = 0.58) than were the yearly rankings for other FHB parameters. Earlier reports on the similarity of cultivar rankings on the basis of INC were inconclusive. Groth et al. (1999), by using a coefficient of determination (r^2) between the INC values for cultivar evaluations across different years, concluded that their results were repeatable. On the other hand, Christensen et al. (1929) and Hanson et al. (1950) reported low repeatability of INC. For our results, the lower values for Spearman rank correlation coefficients for INC than for the other parameters reflect variability among the different environments in which the cultivars were tested and indicate that all cultivars tested had high degree of susceptibility to initial infection, which was most evident under environments with high disease pressure.

Table 3. Spearman's rank correlation coefficients for year-to-year cultivar rankings and Kendall's parameter of concordance (W) from Crookston and St. Paul of four FHB parameters, incidence (INC), disease index (DIS), visually scabby kernels (VSK), and deoxynivalenol (DON).

Location	Years	INC	DIS	VSK	DON
Crookston	1999–2000	0.65*	0.82***	0.87***	0.82***
	1999–2001	0.45	0.66*	0.89***	0.75**
	1999–2002	0.77**	0.64*	0.80**	0.75**
	2000–2001	0.35	0.62*	0.78**	0.49
	2000–2002	0.88***	0.83***	0.71*	0.73**
	2001–2002	0.35	0.56*	0.85***	0.61*
Spearman's rank mean		0.58	0.69	0.82	0.69
Kendall's W		0.65	0.74	0.85	0.74
St. Paul	1999–2000	0.20	0.37	0.57*	0.79**
	1999–2001	0.32	0.34	0.49	0.84***
	1999–2002	0.37	0.60*	0.78**	0.48
	2000–2001	0.89***	0.77**	0.91***	0.75**
	2000–2002	0.48	0.56*	0.60*	0.74**
	2001–2002	0.59*	0.57*	0.48	0.37
Spearman's rank mean		0.48	0.54	0.64	0.66
Kendall's W		0.57	0.62	0.70	0.72

* Significant at 0.05 probability level for year-to-year Spearman's rank correlation coefficients.
** Significant at 0.01 probability level for year-to-year Spearman's rank correlation coefficients.
*** Significant at 0.001 probability level for year-to-year Spearman's rank correlation coefficients.

Table 4. Mean squares from the analyses of variance of the FHB parameters disease index (DIS) and visually scabby kernels (VSK) for 14 wheat cultivars across eight environments.

Source	Disease index (DIS)	Visually scabby kernels (VSK)
Cultivars	10.58**	2 996**
Environments	9.38**	5 295**
Linear regression	63.1**	36 933**
Residual	0.43	22
Cultivars × environments	0.68**	164**
Heterogeneity of regressions	0.96**	485**
Residual	0.63**	109**
Pooled Error	0.25	57

** Significant at 0.01 probability level.

Interestingly, cultivar rankings from the Crookston location (colonized-grain inoculum) were more similar across years than were those from the St. Paul location (macroconidial-spray inoculum), on the basis of the year-to-year Spearman correlations and on Kendall's coefficient of concordance in Table 3. This was not expected because of the greater control over inoculum application provided by the macroconidial spray. The factors responsible for the lower similarity of the cultivar rankings at the St. Paul location are not known but likely include yearly variation in temperature and possibly in precipitation during the period from inoculation to harvest. Variation in precipitation is less likely because of the mist irrigation applied.

Cultivar Stability

Analyses of variance of cultivars across environments (locations × years) for DIS and VSK revealed significant differences among cultivars, environments, and their interactions (C × E) (Table 4). Significant differences among environments indicate that the cultivars were exposed to and evaluated at significantly different disease levels. The linear regression of cultivars on the environmental index was significant and accounted for >95% and 99% of the environmental variance for DIS and VSK, respectively. Heterogeneity of regression was also significant and accounted for about 20 and 43% of the C × E interaction variance for DIS and VSK, respectively. Significant heterogeneity of regression indicates statistical differences in the slopes of the regression lines. The residual of the C × E was still significant and indicates that some other factor(s) besides differences in the slopes of the regression lines, such as deviation from regressions are contributing to the C × E.

The cultivar mean values and stability parameters for DIS and VSK are given in Table 5. Lin et al. (1986) describe three different types of stability concepts: Type 1 stability is possessed by a genotype if its among-environment variance is small; Type 2, if its response to environments parallels the mean response of all cultivars in the trial; and Type 3, if its residual MS from the regression

Table 5. Cultivar means and four stability parameters: regression coefficient b_i (Finlay and Wilkinson, 1963), deviation from regression parameter δ_i^2 (Eberhart and Russell, 1966), and stability variances σ_i^2 and s_i^2 (Shukla, 1972) for Fusarium head blight disease index (DIS) and percent visually scabby kernels (VSK), data from eight environments.

	Disease index (DIS)					Visually scabby kernels (VSK)				
Cultivar†	Mean‡	b_i	δ_i^2	σ_i^2	s_i^2	Mean‡	b_i	δ_i^2	σ_i^2	s_i^2
BacUp	1.4	0.70	0.62	0.65*	0.23	9.6	0.60*	88	154*	34
Forge	1.9	1.70*	1.06**	1.42**	0.42	18.9	1.43*	86	139*	25
Ingot	1.9	1.95**	0.59	1.22**	0.22	11.7	0.70*	51	86	20
2375	2.0	0.36*	0.13	0.42	0.04	16.5	0.80	73*	97	33
Gunner	2.1	1.22	0.27	0.25	0.09	13.3	0.76*	24	46	9
McVey	2.1	1.12	0.41	0.34	0.13	19.8	0.92	63	45	18
Russ	2.2	0.90	0.79	0.70*	0.27	24.5	1.61**	93	216	23
Verde	2.5	1.03	0.94**	0.88*	0.35	23.5	0.64*	29	89	14
HJ98	2.7	1.18	0.26	0.22	0.08	25.4	0.90	153	160*	62
Marshall	2.7	0.64	0.42	0.48	0.15	27.6	0.87	94	121	46
Oxen	2.9	1.09	0.33	0.29	0.12	28.8	1.50**	71	167*	24
Norm	3.1	0.27	0.38	0.42	0.10	44.2	0.52*	154**	225***	78
Roblin	3.4	1.22	1.29**	1.24**	0.47	30.4	1.89***	157	461***	45
Wheaton	3.8	0.53	0.70	0.82**	0.25	49.8	0.71	314**	297***	102

* Significantly different from 1.00 for b_i and from 0.00 for δ_i^2, σ_i^2, and s_i^2 at 0.05 probability level.
** Significantly different from 1.00 for b_i and from 0.00 for δ_i^2, σ_i^2, and s_i^2 at 0.01 probability level.
*** Significantly different from 1.00 for b_i and from 0.00 for δ_i^2, σ_i^2, and s_i^2 at 0.001 probability level.
† Cultivars are ordered from lowest to highest mean DIS.
‡ Mean DIS is on a 0-to-5 scale and VSK is in $\mu g\ g^{-1}$.

model on the environmental index is small. Type 1 stability is not of interest for our analysis of variety response in these disease nurseries. Finlay and Wilkinson's (1963) b_i represents Type 2 stability and stable genotypes are characterized by $b_i = 1$. Eberhart and Russell's (1966) δ_i^2 considers a cultivar stable if the residual mean square from Finlay and Wilkinson's (1963) regression model is not significantly different from zero and it represents Type 3 stability. Shukla's (1972) σ_i^2 and s_i^2 also represent Type 2 stability but are differentiated from Finlay and Wilkinson's b_i because they (σ_i^2 and s_i^2) are derived from the C × E sums of squares instead of the regression coefficient (b_i).

Ideally, resistant cultivars should possess low mean values for parameters of disease response and very low (and nonsignificant) values for b_i and δ_i^2, σ_i^2, and s_i^2. Shukla's σ_i^2 represents the portion of the total C × E variance that is attributable to the ith genotype. On the other hand, s_i^2 represents the portion of only the residual component of the C × E variance that is attributable to the ith genotype. Shukla's s_i^2 is an extension of the model to calculate σ_i^2 and takes into account the covariate z_j (environmental index). If some genotypes show low stability on the basis of σ_i^2 and are judged as stable after taking the covariate into account (as expressed by the significance of s_i^2), it may be inferred that the instability was introduced by the linear effect of the covariate (Shukla, 1972). Seven cultivars in our study show low stability on the basis of σ_i^2 and all became stable after taking the covariate into account. Our results show that BacUp would be a good resistant check because of its high level of resistance and high stability (Table 5). Norm and Wheaton had high mean values for DIS and VSK and low b_i, indicating that they are stable susceptible checks. Three lines (Forge, Roblin, and Verde) had deviations from regression (δ_i^2) values significantly greater than zero for DIS, indicating low stability; however, they were stable on the basis of the other stability parameters. For both DIS and VSK (Table 5), and for INC (data not shown), results from stability analyses revealed stability for FHB response in resistant, intermediate, and susceptible cultivars. Our results differ from those of Mesterházy (1995) who concluded that stability for disease reaction was correlated with resistance level, with the most resistant cultivars being most stable and the most susceptible cultivars being less stable. Although different, our results do not necessarily contradict those of Mesterházy (1995). Mesterházy (1995) included factors that led to cases of symptomless susceptible cultivars, e.g., when conditions were less favorable for disease development and when the fungal isolate tested was a weak pathogen. Therefore, the susceptible cultivars showed few visible symptoms of disease in some environments but suffered devastating damage in others. Our tests were done under different environmental conditions but all with disease levels sufficient to differentiate resistant from susceptible cultivars.

Resource Allocation for FHB Screening

An approach to decide on practical limits for subsampling, number of replications, and number of environments is to estimate genotype standard error (SE) under varied numbers of these parameters. Standard error $= [(s_\varepsilon^2 + r s_{CE}^2)/re]^{1/2}$, where r = number of replications, e = number of environments, and other terms as described previously. Resources should be allocated such that for any given effort (resources used), the genotype standard error is minimized. Lower genotype standard errors maximize the probability of finding significant differences among genotypes and give greater confidence that a genotype's FHB reaction has been correctly characterized. Increasing the number of environments from one to two or one to three at any given level of replication or spikes per plot analyzed resulted in the greatest reduction in genotype standard error for DIS compared with increasing either the number of replications or spikes per plot (Table 6). Campbell and Lipps (1998) compared different sources of variability on FHB response and found that the magnitude of the within-

Table 6. Estimated genotype standard errors (SE)† for Fusarium head blight disease index (DIS) among 14 wheat cultivars under differing levels of subsampling plot^{-1} (spike number), plot replication within environments, and environments.

	Replications with 10 spikes plot^{-1}					Replications with 20 spikes plot^{-1}					Replications with 30 spikes plot^{-1}				
No. of env.	2	3	4	6	8	2	3	4	6	8	2	3	4	6	8
1	0.52	0.48	0.45	0.43	0.42	0.50	0.46	0.44	0.42	0.41	0.49	0.46	0.44	0.42	0.41
2	0.37	0.34	0.32	0.30	0.29	0.35	0.33	0.31	0.30	0.29	0.35	0.32	0.31	0.30	0.29
3	0.30	0.27	0.26	0.25	0.24	0.29	0.27	0.26	0.24	0.24	0.28	0.26	0.25	0.24	0.24
4	0.26	0.24	0.23	0.21	0.21	0.25	0.23	0.22	0.21	0.21	0.25	0.23	0.22	0.21	0.20
6	0.21	0.19	0.18	0.18	0.17	0.20	0.19	0.18	0.17	0.17	0.20	0.19	0.18	0.17	0.17
8	0.18	0.17	0.16	0.15	0.15	0.18	0.16	0.16	0.15	0.15	0.17	0.16	0.15	0.15	0.14
10	0.16	0.15	0.14	0.14	0.13	0.16	0.15	0.14	0.13	0.13	0.16	0.14	0.14	0.13	0.13

† SE = $[(s^2_{\hat{\varepsilon}} + rs^2_{CE})/re]^{1/2}$, where r = number of replications, e = number of environments; $s^2_{\hat{\varepsilon}}$ (plot error variance) = $s^2_w/n + s^2_b$, where s^2_w is the within-plot variance with n spikes plot^{-1} evaluated, s^2_b is between plot variance, and s^2_{CE} is the estimated cultivar × environment variance. Estimated variances are as follows: $s^2_w = 0.7927$; $s^2_b = 0.1713$; $s^2_{CE} = 0.1424$.

plot variance was so high that it impeded detection of significant differences among lines tested. They used estimates of standard errors to make recommendations regarding resource allocation. They obtained the largest reduction in SE by increasing the number of environments, then replications. Compared to the cost of evaluating one additional spike per plot, an additional replication or environment cost 10 or 50 times more, respectively (Campbell and Lipps, 1998), and total costs were optimized if eight spikes per plot were evaluated in four replications per environment. The authors made no specific recommendation regarding the appropriate number of environments to assess for allocation of resources when screening for FHB reaction although they noted that three or more environments required a total cost per genotype that exceeded their resources (Campbell and Lipps, 1998).

Our results lead us to disagree with such a resource allocation. While the net cost of adding one environment may be higher than that of adding a replication, reducing replication number from four to two would reduce an environment to half its size. It is important to realize that the number of plots to be evaluated at any given location-year (environment) may be limited by factors such as irrigation capacity, labor for inoculation and scoring entries, etc. Increasing the number of environments from one with four replications to two environments with two replications each would minimize the risks of losing the entire location due to adverse conditions. In addition, it will lower estimates of genotype standard error. In our study, the estimated value of genotype standard error is 0.44 when evaluating either 20 or 30 spikes per plot in four replications in one environment. The genotype standard error is reduced to 0.35 when evaluating either 20 or 30 spikes per plot in two environments with two replications each. Carter et al. (1983), working with soybean [*Glycine max* (L.) Merr.], concluded that, because of the presence of G × E, one should expect to test in multiple environments for a reliable ranking of treatments and recommended testing in at least two environments and at least seven environments to detect 20 and 10% of treatment differences, respectively. Mesterházy (1995) recommended evaluating lines in at least three environments before making conclusions with regard to their FHB response. We predicted $LSD_{0.05}$ for DIS for spike numbers of 10, 20, and 30 per plot and replicate numbers of 2, 3, 4, 6, and 8 in 1, 2, 3, 4, 6, 8, and 10 environments (Table 7). We propose that $LSD_{0.05}$ magnitudes of approximately 33%, or less, of the observed range of values are sufficient for finding important differences. The difference between extreme cultivars for DIS was 2.4, calculated from across-environment means (Table 1). Accordingly, an $LSD_{0.05}$ of less than 0.8 is suggested. Three environments and three replicates with 10 spikes achieve this goal for a total of 90 spikes evaluated for each genotype. Our results suggest that in breeding spring wheat for FHB resistance, the number of replications per environment beyond three and the number of spikes per plot beyond 10 have little practical value and reduces efficiency for FHB research.

CONCLUSIONS

Both colonized-grain and conidial-spray inoculation methods provide disease levels appropriate to differentiate resistant and susceptible cultivars. When breeding for FHB resistance, it is imperative to evaluate lines with

Table 7. Predicted least significant differences [LSD (P = 0.05)]† for Fusarium head blight disease index (DIS) among 14 wheat cultivars under differing levels of subsampling plot^{-1} (spike number), plot replication within environments, and environments.

	Replications with 10 spikes plot^{-1}					Replications with 20 spikes plot^{-1}					Replications with 30 spikes plot^{-1}				
No. of env.	2	3	4	6	8	2	3	4	6	8	2	3	4	6	8
1	1.58	1.45	1.38	1.31	1.27	1.52	1.41	1.35	1.29	1.25	1.50	1.39	1.34	1.28	1.25
2	1.12	1.03	0.98	0.93	0.90	1.08	1.00	0.95	0.91	0.89	1.06	0.99	0.95	0.90	0.88
3	0.87	0.80	0.76	0.72	0.70	0.84	0.77	0.74	0.71	0.69	0.82	0.77	0.74	0.70	0.69
4	0.74	0.68	0.65	0.61	0.60	0.71	0.66	0.63	0.60	0.59	0.70	0.65	0.63	0.60	0.58
6	0.60	0.55	0.52	0.49	0.48	0.57	0.53	0.51	0.49	0.47	0.57	0.53	0.50	0.48	0.47
8	0.51	0.47	0.45	0.43	0.41	0.49	0.46	0.44	0.42	0.41	0.49	0.45	0.43	0.42	0.41
10	0.46	0.42	0.40	0.38	0.37	0.44	0.41	0.39	0.37	0.36	0.43	0.40	0.39	0.37	0.36

† LSD = $t_{(0.05,\,df)} \times [2 \times (s^2_{\hat{\varepsilon}} + rs^2_{CE})/re]^{1/2}$, where df = degrees of freedom for pooled error, r = number of replications, e = number of environments; $s^2_{\hat{\varepsilon}}$ (plot error variance) = $s^2_w/n + s^2_b$, where s^2_w is the within-plot variance with n spikes plot^{-1} evaluated, s^2_b is between plot variance, and s^2_{CE} is the estimated cultivar × environment variance. Estimated variances are as follows: $s^2_w = 0.7927$; $s^2_b = 0.1713$; $s^2_{CE} = 0.1424$.

resistant and susceptible check cultivars known to be stable in their FHB response. Stability of FHB reactions was not associated with the level of resistance in the cultivars tested. Increasing the number of environments was most effective in reducing the genotype standard error and therefore increasing the probability of finding significant differences among genotypes evaluated. We recommend that wheat breeding programs that test a large number of near-homozygous, early-generation lines (e.g., F_4–F_6 derived) initially use one or two environments to identify and discard highly susceptible lines. Selected lines should continue to be evaluated in subsequent FHB trials to assess more accurately their response across more environments. A good assessment of cultivar FHB reaction can be obtained in three or four different environments provided that disease pressure is sufficient for differentiating resistant from susceptible lines.

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